

# TECHNICAL MEMORANDUM

CH2MHILL

**gdc**

## BIOASSAY TESTING – JOINT CANNERY OUTFALL EFFLUENT AUGUST 2000 SAMPLING

**Prepared For:** StarKist Samoa (NPDES Permit AS0000019)  
COS Samoa Packing (NPDES Permit AS0000027)

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**Date:** 18 October 2000

**Distribution:** Carl Goldstein  
United States Environmental Protection Agency, Region 9  
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American Samoa Environmental Protection Agency

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### ***Purpose***

This memorandum presents the results of the bioassay testing of the Joint Cannery Outfall effluent sample that was collected in August 2000. This is the 14<sup>th</sup> required semi-annual test. Separate technical memoranda have been being prepared to describe the results of concurrent effluent chemistry testing.

### ***Study Objectives***

Section D.1 of the StarKist Samoa and COS Samoa Packing NPDES permits requires that semi-annual definitive acute bioassays (96-hour static bioassays) be conducted on the cannery effluent. The purpose of these tests is to determine whether, and at what effluent concentration, acute toxicity may be detected for the effluent.

U.S. EPA has conducted a number of reviews of the effluent sampling, analysis, and bioassay tests. All comments from U.S. EPA have been incorporated into either the

Standard Operating Procedures or have been incorporated into the procedures used by the laboratory doing the test, Advanced Biological Testing, Inc., as documented in previous reports.

The bioassay tests were originally specified using the white shrimp, *Penaeus vannamei* (postlarvae). In the event *Penaeus vannamei* is not available at the time of the tests, a substitute species, *Mysidopsis bahia*, has been approved by U.S. EPA (CH2M HILL, 26 January 1995). For the August 2000 sampling, *Penaeus vannamei* was not available and *Mysidopsis bahia* was used.

The effluent acute bioassay sampling must be concurrent with effluent sampling for chemical analysis. Effluent samples were collected as 24-hour composite samples. The effluent acute bioassay was conducted using a combined composite effluent sample made up from the composite effluent samples from the StarKist Samoa and COS Samoa Packing facilities, as approved by EPA. This combined effluent bioassay is representative of the wastewater discharged from the joint cannery outfall to Pago Pago Harbor.

### ***Effluent Sampling Methods***

Between 1200 on 24 August 2000 and 0900 on 25 August 2000, 24-hour, flow-weighted, composite samples of final effluent were collected from both the StarKist Samoa and COS Samoa Packing effluent discharges. Samples were collected from the established effluent sampling sites following the routine composite sample collection schedule for the plants. Detailed sampling procedures are described in the Standard Operating Procedures (SOP) which was provided in Attachment I of the report *Bioassay Testing – Joint Cannery Outfall Effluent February 2000 Sampling*, CH2M HILL and gdc, 18 April 2000.

A total of eight grab samples were collected into pre-cleaned 1-gallon plastic cubitainers at each plant. Samples were collected at approximately three-hour intervals over a 24-hour period. The samples were stored on ice until the completion of the 24-hour sampling period. After all samples were collected a flow-proportioned composite sample was prepared. The grab sample collection times and the relative effluent volumes calculated from plant flow records are summarized in Table 1. The relative effluent volumes were used to prepare the final composite sample, which was used to fill the sample container shipped to the laboratory for testing.

A 5-gallon cubitainer containing the composite sample was packed on ice in an ice chest for shipment to the laboratory. A chain-of-custody form for the sample was completed and then sealed into a zip-lock bag and taped inside the lid of the ice chest. The sample was shipped via DHL on flights from Pago Pago to Honolulu and then to San Francisco. The testing laboratory received the sample on 28 August 2000. The chain-of-custody form is provided in Attachment I.

### ***Bioassay Testing Procedures***

The bioassay tests were conducted by Advanced Biological Testing Inc., Rohnert Park, California. The testing procedures and results of the bioassay tests are provided in the Laboratory report included as Attachment II. This report summarizes the 96-hour acute bioassay test conducted with reference to U.S. EPA document EPA/600/4-90/027F, August 1993, as the source of methods for conducting the test.

The bioassay test was conducted considering and including U.S. EPA's comments on previous bioassay tests, as documented in previous reports. A brine control was run and a comparison was made with the dilution water laboratory control. The test organisms were required to be 1 to 5 days old, with a 24-hour range in age, and the test temperature was to be held at  $20 \pm 1^{\circ}\text{C}$  or  $25 \pm 1^{\circ}\text{C}$ . For this bioassay, two-day-old *Mysidopsis bahia* were used.

Because of the demonstrated potential for a lethal immediate dissolved oxygen demand (IDOD), discussed and documented in previous technical memoranda describing the first two bioassay tests, each bioassay test chamber was continuously aerated during the bioassay tests to maintain adequate levels of dissolved oxygen (DO). Bioassay tests were carried out for effluent concentrations of 50, 25, 12.5, 6.25, and 3.1% as vol:vol dilutions in seawater. Water quality was monitored daily and parameters measured included DO, pH, salinity, temperature, and ammonia. Additionally, a reference toxicant of sodium dodecyl sulfonate (SDS) was made up of a 2-gram per liter stock solution in distilled water and tested at concentrations of 25, 12.5, 6.25, 3.1, and 1.9 mg/L in 31 ppt seawater for a 96-hour test.

## **Results**

The results of the bioassay tests are summarized as follows:

***Mysidopsis bahia* Effluent Bioassay.** All results from the bioassay tests are included in Attachment II. The results of the mysid bioassay tests indicate the LC<sub>50</sub> for the effluent tested was 17.1 percent. The No Observable Effects Concentration (NOEC) for the 96-hour bioassay was 3.1 percent and the Least Observable Effects Concentration (LOEC) was 6.25 percent.

***Mysidopsis bahia* Reference Toxicant Bioassay.** The reference toxicant test had an LC<sub>50</sub> of 17.9 mg/l. The laboratory mean is 14.71±4.10 mg/l. The test data falls within one standard deviation of the laboratory reference mean, indicating normal sensitivity.

## **Discussion**

Table 2 summarizes the results of the effluent bioassay tests for the samples collected in the August 2000 sampling compared to the previous bioassay tests. The LC<sub>50</sub>, NOEC and LOEC are within the range obtained from previous reports where *Mysidopsis bahia* was used in place of *Penaeus vannamei*.

## **Conclusions**

The bioassay tests for the Joint Cannery Outfall effluent for August 2000 do not indicate effluent toxicity levels to be of concern. As discussed in the previous bioassay test reports on the effluent, the time scale of the mixing of the effluent with the receiving water is on the order of minutes to seconds to achieve dilutions that will eliminate possible toxic effects as reflected by the bioassay results. For example, an NOEC of 3.1% which was observed in August 2000, corresponds to a dilution of 32:1 which is achieved within a time frame of seconds and within a few meters of the discharge point. The discharge is located in about 180 feet of water and the effluent toxicity tests indicate that the discharge is diluted to non-toxic levels immediately after discharge and well within the initial dilution plume.

BIOASSAY TESTING – JOINT CANNERY OUTFALL EFFLUENT  
AUGUST 2000 SAMPLING

**Table 1**  
**StarKist Samoa and COS Samoa Packing 24-hour Composite Effluent**  
**Sample for Bioassay Testing**  
**August 2000**

Grab Sample Number	COS Samoa Packing		StarKist Samoa		COS Samoa Packing Percent of Total Flow	StarKist Samoa Percent of Total Flow
	Sampling Date and Time	Effluent Flow Rate (mgd)	Sampling Date and Time	Effluent Flow Rate (mgd)		
	<u>24 Aug 2000</u>		<u>24 Aug 2000</u>			
1	1200	0.44	1200	1.29	2.8	8.1
2	1500	0.48	1500	1.37	3.0	8.7
3	1800	0.56	1800	1.47	3.5	9.3
4	2100	0.56	2100	1.61	3.5	10.1
5	2400	0.44	2400	1.47	2.8	9.3
	<u>25 Aug 2000</u>		<u>25 Aug 2000</u>			
6	0300	0.56	0300	1.59	3.5	10.0
7	0600	0.52	0600	1.67	3.3	10.5
8	0900	0.52	0900	1.32	3.3	8.3
<b>Total</b>		4.08		11.79	25.7	74.3
<b>Mean</b>		0.51		1.47		

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<p style="text-align: center;"><b>Table 2</b> <b>StarKist Samoa and COS Samoa Packing</b> <b>Combined Effluent Bioassay Results</b></p>				
Date	Species	Parameters		
		LC 50	NOEC	LOEC
2/93	<i>Penaeus vannamei</i>	4.8% <sup>1</sup>	3.1%	6.25%
10/93	<i>Penaeus vannamei</i>	15.67%	3.1%	6.25%
2/94	<i>Penaeus vannamei</i>	15.76%	<1.6%	1.6%
10/94	<i>Mysidopsis bahia</i> <sup>2</sup>	31.2%	25%	50%
3/95	<i>Penaeus vannamei</i>	14.8%	6.25%	12.5%
3/95	<i>Mysidopsis bahia</i> <sup>3</sup>	10.8%	6.25%	12.5%
2/96	<i>Penaeus vannamei</i>	>50%	>50%	>50%
2/96	<i>Mysidopsis bahia</i> <sup>3</sup>	28.36%	12.5%	25%
3/96	<i>Penaeus vannamei</i>	44.4%	25%	50%
11/96	<i>Penaeus vannamei</i>	7.11%	3.1%	6.25%
03/97	<i>Penaeus vannamei</i>	39.36%	12.5%	25%
09/97	<i>Penaeus vannamei</i> <sup>4</sup>	12.3%	6.25%	12.5%
06/98	<i>Mysidopsis bahia</i> <sup>2</sup>	17.2%	6.25%	12.5%
11/98	<i>Mysidopsis bahia</i> <sup>2</sup>	15%	6.25%	12.5%
02/00	<i>Mysidopsis bahia</i> <sup>2</sup>	20%	6.25%	12.5%
08/00	<i>Mysidopsis bahia</i> <sup>2</sup>	17.1%	3.1%	6.25%
<p><sup>1</sup>The February 1993 samples were not aerated until after the first day of the test. For subsequent tests the samples were aerated for the entire duration of the tests.</p> <p><sup>2</sup><i>Mysidopsis bahia</i> used as substitutes because <i>Penaeus vannamei</i> not available: as directed and approved by U. S. EPA.</p> <p><sup>3</sup><i>Mysidopsis bahia</i> used in addition to <i>Penaeus vannamei</i> as described in text of technical memorandums reporting test results. Only one species is required by the permit conditions.</p> <p><sup>4</sup>Stage 1 (3 mm) <i>Penaeus vannamei</i> were used for testing because older Stage 7 and 8 (8-10 mm) <i>Penaeus vannamei</i> were not available.</p>				

**ATTACHMENT I**

**CHAIN-OF-CUSTODY FORM**

**JOINT CANNERY OUTFALL EFFLUENT SAMPLE**

**COS Samoa Packing Company, Inc.**

**and**

**StarKist Samoa, Inc.**

**August 2000**

**CH2MHILL Applied Sciences Lab**  
CHAIN OF CUSTODY RECORD  
AND AGREEMENT TO PERFORM SERVICES

CVD 2300 NW Walnut Boulevard  
Corvallis, OR 97330-3638  
(541) 752-4271 FAX (541) 752-0278

[illegible]

**Instructions and Agreement Provisions on Reverse Side**

**DISTRIBUTION:** Original - LAB, Yellow - LAB, Pink - Client

10-16-2000 10:14AM FROM ADV BIO TEST INC 7075882884

12.



**ATTACHMENT II**

**LABORATORY REPORT  
Advanced Biological Testing  
96-hour Acute Bioassay**

**JOINT CANNERY OUTFALL EFFLUENT SAMPLE  
COS Samoa Packing Company, Inc.  
and  
StarKist Samoa, Inc.**

**August 2000**

**RESULTS OF BIOASSAYS CONDUCTED ON  
AN EFFLUENT SAMPLE  
FROM THE JOINT CANNERY OUTFALL  
IN AMERICAN SAMOA  
Using *Mysidopsis bahia***

Prepared for:

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September 13, 2000

Ref: 2018

## INTRODUCTION

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At the request of CH2M Hill (Project # 147323.JC.BA), Advanced Biological Testing conducted a four day effluent bioassay test on *Mysidopsis bahia* using effluents collected from the joint cannery outfall at the Starkest and Van Camp tuna canneries in American Samoa. The studies were run using methods generally specified in EPA 1991. *Penaeus* is the preferred species according to the NPDES permit, however when *Penaeus* are unavailable, *Mysidopsis* has been substituted. *Penaeus* was not available to start this test and *Mysidopsis* was used instead.

The study was conducted at the Advanced Biological Testing Laboratory in Rohnert Park, California, and was managed by Mr. Mark Fisler.

## METHODS

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### 2.1 EFFLUENT SAMPLING

The effluents were sampled on August 24, 2000 by cannery personnel under the supervision of CH2M Hill. The sample was received by the laboratory on August 28, 2000. One five gallon carboy was provided and maintained in an ice-filled cooler from the date of sampling until laboratory receipt. The sample was at 5°C upon receipt.

### 2.2 SAMPLE PREPARATION

The salinity of the effluent sample was 16.1 ppt and required salinity adjustment to 27 ppt. The effluent salinity was increased to 27 ppt with 100 ppt natural seawater brine. The brine was made from frozen Bodega Bay seawater. Due to the dilution of the effluent with the brine solution, the initial maximum concentration of effluent was 87.0%. The highest initial test concentration was made by diluting the 87% effluent with Bodega Bay seawater to an actual effluent concentration of 50%. The dissolved oxygen level in the sample was low. The initial total ammonia was approximately 7.2 ppm (3.57 ppm in the 50% test sample).

The effluents were tested at an actual effluent concentration series of 50%, 25%, 12.5%, 6.25%, and 3.1% as vol:vol dilution in seawater. A brine control was run with the test to assess the potential toxicity from the added brine. The diluent and the control water were filtered seawater from Bodega Bay. The dilutions were brought to the test temperature ( $20 \pm 2^{\circ}\text{C}$ ) and aerated continuously. Based upon the previous testing, these effluents have an increasing biological oxygen demand, with a significant peak at 10-14 hours after test initiation. Previous testing of this effluent conducted without initial aeration demonstrated significant toxicity at 24 hours (or before); therefore aeration was carried out from the beginning of the test. According to EPA methods the test chambers were renewed with retained effluents held under refrigeration from test initiation on Day 2.

A reference toxicant was run using concentrations initially provided by the EPA. The toxicant was sodium dodecyl sulfonate (SDS) made up as a 1 grams per liter stock solution in distilled water. The tested concentrations were set at 50, 25, 12.5, 6.25, and 3.1 mg/L in 27 ppt seawater.

### 2.3 TESTING PROCEDURES

The bioassays were carried out on two day old larvae of *Mysidopsis bahia* supplied by Aquatox in Arkansas. The mysids were received on August 29, 2000 and were used immediately. Five replicates of each concentration were tested with ten animals per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

### 2.4 STATISTICAL ANALYSIS

At the conclusion of the test, the survival data were evaluated statistically using statistical programs designed to determine ECp, NOEC, and LOEC values where appropriate. These comprehensive statistical applications follow standard guidelines for acute toxicity data analysis. Statistical effects can be measured by the ECp, the estimated concentration that causes any effect, either lethal (LC) or sublethal (IC), on p% of the test population. The LCp is the point estimate of the concentration at which a lethal effect is observed in p% of the test organisms. ECp values include 95% confidence limits if calculable.

## RESULTS

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### 3.1 INTRODUCTION

Tables 1 through 6 present the results of the *Mysidopsis* testing. The test conditions are summarized in Table 1. In the test, water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at  $20 \pm 2^{\circ}\text{C}$ ; the pH remained relatively stable, and the salinity increased very slightly as would be expected in a static test (Tables 2 and 3). Aeration was maintained in all chambers for the duration of the test. The test solutions were renewed with reserved effluent at 48 hrs.

Initial ammonia was 3.57 ppm in the 50% effluent and was proportionally diluted at lower percentage concentrations. The LC50 for the effluent was 17.1. There was significant mortality at the 6.25%, 12.5%, 25% and 50% concentrations compared to the control (Table 4). The NOEC was 3.1%, and the LOEC was 6.25%. The TU was 32.

The reference toxicant test had an LC50 of 17.9 mg/L (Tables 5 and 6). The laboratory mean for *Mysidopsis bahia* was  $14.71 \text{ mg/L} \pm 4.10 \text{ mg/L}$ . The data is within one standard deviation of the laboratory mean, indicating normal sensitivity.

Advanced Biological Testing Inc.

TABLE 1  
Bioassay Procedure And Organism Data  
For the Survival Bioassay  
Using *Mysidopsis bahia*(U.S. EPA 1991)

Parameter	Data
<b><u>Sample Identification</u></b>	
Sample ID(s)	000828-1
Date Sampled	8/24/00
Date Received at ABT	8/28/00
Volume Received	Five gallons
Sample Storage Conditions	4°C in the dark
<b><u>Test Species</u></b>	
Supplier	Aquatox, Hot Springs, Arkansas
Collection location	In house colony
Date Acquired	8/29/00
Acclimation Time	Used immediately
Acclimation Water	Shipping water
Acclimation Temperature	20±2°C
Age group	Two day old larvae
<b><u>Test Procedures</u></b>	
Type, Duration	Acute, static/renewal at 48 hours
Test Dates	8/29/00-9/2/00
Control Water	Bodega Bay seawater
Test Temperature	20± 2°C
Test Photoperiod	14 L : 10 D
Salinity	27± 2 ppt
Test Chamber	1000 mL jars
Animals/Replicate	10
Exposure Volume	500 mL
Replicates/Treatment	5
Feeding	Brine shrimp (<24 hr old nauplii)
Deviations from procedures	None

Table 2

**INITIAL WATER QUALITY MEASUREMENTS**  
**For Acute Mysid Effluent Test**  
**Test Dates: 8/29/00 - 9/2/00**

Concentration (%)	Day 0					Day 2				
	pH	DO	NH 3	°C	Sal	pH	DO	NH 3	°C	Sal
<b>Control</b>	8.20	7.7	0.01	19.1	27	7.88	7.2	0.12	19.3	27
<b>Brine</b>	8.17	7.7		18.8	27	8.06	7.0		19.2	27
<b>3.1</b>	8.10	7.8		19.1	27	8.13	7.1		19.1	28
<b>6.25</b>	8.02	7.7		19.2	27	8.13	7.0		19.1	28
<b>12.5</b>	7.82	7.6		19.2	27	8.10	7.0		19.1	28
<b>25</b>	7.50	7.2		19.3	27	8.02	6.5		19.1	28
<b>50</b>	7.22	6.4	3.57	19.3	27	7.88	3.7	4.21	18.8	28
<b>Min</b>	7.22	6.4	0.01	18.8	27	7.88	3.7	0.12	18.8	27
<b>Max</b>	8.20	7.8	3.57	19.3	27	8.13	7.2	4.21	19.3	28



Table 3

## Final Water Quality Measurements For the Acute Mysid Effluent Test

Concentration (%)	Rep	Day 1				Day 2				Day 3				Day 4			
		pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal
Control	1	8.34	7.3	18.5	28	8.16	7.5	18.9	28	8.22	8.0	18.9	28	8.18	7.9	19.1	28
	2	8.34	7.4	18.5	28	8.20	7.6	18.9	29	8.23	8.0	18.8	28	8.20	7.9	19.0	28
	3	8.30	7.4	18.4	28	8.16	7.5	18.7	28	8.20	8.0	18.8	28	8.16	7.8	18.9	29
	4	8.33	7.6	18.3	28	8.23	7.6	18.7	28	8.24	8.0	18.7	28	8.26	7.8	18.7	28
	5	8.34	7.6	18.3	28	8.24	7.6	18.6	28	8.27	8.0	18.6	28	8.27	7.8	18.6	28
Brine	1	8.34	7.7	18.3	27	8.24	7.7	18.6	28	8.27	8.1	18.7	28	8.27	8.0	18.6	28
	2	8.35	7.7	18.4	27	8.27	7.7	18.7	28	8.29	8.1	18.8	28	8.28	8.0	18.9	28
	3	8.36	7.7	18.4	27	8.28	7.7	18.7	27	8.30	8.0	18.8	28	8.29	8.0	18.9	28
	4	8.33	7.7	18.3	27	8.22	7.6	18.7	27	8.25	8.0	18.8	28	8.26	8.0	18.8	28
	5	8.33	7.7	18.4	27	8.24	7.6	18.7	27	8.26	8.0	18.7	28	8.26	8.0	18.7	28
3.1	1	8.32	7.7	18.3	28	8.25	7.7	18.7	28	8.25	8.0	18.7	28	8.27	8.0	18.9	29
	2	8.28	7.7	18.5	27	8.27	7.7	18.8	28	8.29	8.0	18.8	28	8.29	8.0	19.0	29
	3	8.26	7.7	18.4	27	8.18	7.7	18.8	28	8.23	7.9	18.8	28	8.21	7.9	19.0	28
	4	8.26	7.7	18.4	27	8.18	7.6	18.7	28	8.21	7.9	18.8	28	8.22	7.9	18.9	28
	5	8.25	7.7	18.4	27	8.18	7.6	18.8	28	8.20	7.9	18.8	28	8.22	7.9	18.9	28
6.25	1	8.25	7.7	18.4	28	8.16	7.6	18.8	28	8.24	7.8	18.7	28	8.26	7.8	18.9	29
	2	8.30	7.7	18.4	28	8.24	7.6	18.7	28	8.29	7.8	18.7	28	8.30	7.9	19.1	28
	3	8.29	7.7	18.5	27	8.22	7.6	18.7	28	8.27	7.9	18.7	28	8.30	7.9	19.1	28
	4	8.27	7.7	18.4	28	8.16	7.5	18.7	28	8.21	7.8	18.7	28	8.29	7.9	19.0	28
	5	8.23	7.7	18.5	27	8.25	7.5	18.7	28	8.28	7.8	18.7	28	8.30	7.9	19.0	28
12.5	1	8.36	7.6	18.3	28	8.27	7.6	18.4	29	8.39	7.8	18.5	29	8.38	7.8	18.6	30
	2	8.39	7.7	18.3	28	8.28	7.7	18.6	28	8.41	7.8	18.5	28	8.41	7.9	18.6	28
	3	8.28	7.6	18.3	28	8.20	7.5	18.5	29	8.27	7.5	18.5	28	8.31	7.8	18.6	29
	4	8.36	7.6	18.3	28	8.31	7.5	18.6	28	8.34	7.7	18.5	28	8.37	7.8	18.6	28
	5	8.36	7.6	18.4	28	8.31	7.6	18.6	28	8.33	7.7	18.5	28	8.38	7.9	18.6	28
25	1	8.34	7.6	18.3	28	8.30	7.6	18.6	28	8.35	7.8	18.6	28	8.40	7.8	18.7	28
	2	8.37	7.6	18.3	28	8.37	7.6	18.4	28	8.36	7.7	18.6	28	8.41	7.8	18.7	28
	3	8.43	7.6	18.3	28	8.42	7.6	18.4	28	8.40	7.8	18.5	28	8.47	7.8	18.6	28
	4	8.42	7.7	18.3	28	8.42	7.6	18.4	28	8.40	7.8	18.5	28	8.40	7.8	18.6	28
	5	8.33	7.7	18.3	27	8.36	7.6	18.4	28	—	—	—	—	—	—	—	—
50	1	8.22	6.8	18.2	28	8.29	7.5	18.4	28	8.21	7.7	18.6	28	8.34	7.8	18.7	28
	2	8.27	6.9	18.1	28	8.39	7.5	18.4	28	8.20	7.5	18.5	28	8.26	7.8	18.6	28
	3	8.30	7.0	18.2	28	8.46	7.5	18.3	28	8.27	7.4	18.6	28	8.34	7.6	18.6	29
	4	8.39	7.0	18.1	28	8.42	7.5	18.3	28	8.47	7.5	18.5	28	8.37	7.5	18.7	28
	5	8.17	7.0	18.1	28	8.32	7.4	18.3	28	—	—	—	—	—	—	—	—
Min		8.17	6.8	18.1	27	8.16	7.4	18.3	27	8.20	7.4	18.5	28	8.16	7.5	18.6	28
Max		8.43	7.7	18.5	28	8.46	7.7	18.9	29	8.47	8.1	18.9	29	8.47	8.0	19.1	30

Note: — = All animals dead.

Table 4

## Summary of Results for the Mysid Acute Effluent Test

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	94.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	9	90	
	4	10	10	10	10	10	100	
	5	10	10	10	8	8	80	
Brine	1	10	10	10	10	9	90	98.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
3.1	1	10	10	10	10	9	90	90.0
	2	10	10	10	10	10	100	
	3	10	10	9	9	9	90	
	4	10	10	10	10	10	100	
	5	10	10	10	7	7	70	
6.25	1	10	10	10	10	9	90	78.0
	2	10	10	9	8	7	70	
	3	10	10	8	8	8	80	
	4	10	10	10	9	7	70	
	5	10	10	9	9	8	80	
12.5	1	10	10	7	*	5	50	60.0
	2	10	10	8	*	6	60	
	3	10	10	8	*	6	60	
	4	10	10	5	*	5	50	
	5	10	10	9	*	8	80	
25	1	10	*	8	*	5	50	38.0
	2	10	*	8	*	4	40	
	3	10	*	9	*	6	60	
	4	10	*	8	*	4	40	
	5	10	*	0	—	0	0	
50	1	10	0	1	*	0	0	0.0
	2	10	0	2	*	0	0	
	3	10	10	2	*	0	0	
	4	10	0	1	*	0	0	
	5	10	0	0	—	0	0	

Note: — = All animals dead.

\* = Water too turbid to count.

LC50 = 17.1

Table 5

## Water Quality Measurements for the Mysid Reference Toxicant Bioassay

Concentration (mg/L)	Rep	Day 0				Day 1				Day 2				Day 3				Day 4			
		pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal
Control	1	8.04	7.8	19.4	27	8.30	7.8	19.2	27	8.21	6.4	19.4	28	8.18	6.7	19.4	28	8.26	6.7	19.5	28
	2					8.28	7.8	19.2	27	8.20	6.5	19.5	28	8.17	6.7	19.4	28	8.14	6.5	19.5	28
	3					8.26	7.7	19.2	27	8.16	6.4	19.5	28	8.13	6.6	19.4	28	8.08	6.3	19.5	28
3.1	1	8.02	7.7	19.3	27	8.25	7.6	19.2	27	8.14	6.3	19.5	27	8.13	6.6	19.4	28	8.09	6.0	19.4	28
	2					8.21	7.6	19.2	27	8.10	6.2	19.5	27	8.10	6.5	19.4	28	8.06	6.0	19.5	28
	3					8.17	6.3	19.2	27	8.06	6.2	19.5	27	8.08	6.5	19.4	28	8.04	6.0	19.5	28
6.25	1	8.02	7.7	19.3	27	8.17	6.2	19.2	27	8.05	5.8	19.5	27	8.08	6.5	19.3	28	8.04	6.0	19.5	28
	2					8.14	6.2	19.2	27	8.02	5.8	19.4	27	8.06	6.4	19.3	28	8.02	6.0	19.6	28
	3					8.14	6.1	19.2	27	8.01	5.8	19.5	27	8.08	6.4	19.3	28	8.02	6.0	19.5	28
12.5	1	8.04	7.7	19.3	27	8.14	6.0	19.2	27	7.95	4.4	19.5	27	8.05	6.3	19.3	27	8.02	6.0	19.5	28
	2					8.14	6.0	19.2	27	7.91	4.5	19.4	27	8.04	6.3	19.3	27	8.00	6.0	19.5	28
	3					8.14	6.0	19.2	27	7.87	4.4	19.5	27	8.01	6.3	19.3	27	8.00	6.0	19.5	28
25	1	8.04	7.6	19.3	27	8.14	6.1	19.2	27	7.84	3.7	19.5	27	8.01	3.9	19.3	27	—	—	—	—
	2					8.16	6.1	19.2	27	7.83	3.7	19.5	27	7.93	3.9	19.3	27	8.01	5.7	19.5	27
	3					8.16	6.1	19.2	27	7.80	3.8	19.5	27	7.92	4.0	19.3	27	—	—	—	—
50	1	8.06	7.6	19.3	26	8.17	6.3	19.2	26	—	—	—	—	—	—	—	—	—	—	—	—
	2					8.18	6.3	19.2	26	—	—	—	—	—	—	—	—	—	—	—	—
	3					8.18	6.3	19.2	26	—	—	—	—	—	—	—	—	—	—	—	—
Min		8.02	7.6	19.3	26	8.14	6.0	19.2	26	7.80	3.7	19.4	27	7.92	3.9	19.3	27	8.00	5.7	19.4	27
Max		8.06	7.8	19.4	27	8.30	7.8	19.2	27	8.21	6.5	19.5	28	8.18	6.7	19.4	28	8.26	6.7	19.6	28

Note: — = All animals dead.

Table 6

## Summary of Results for the Mysid Reference Toxicant Bioassay

Concentration (mg/L)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
3.1	1	10	9	9	9	9	90	96.7
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
6.25	1	10	10	10	10	9	90	93.3
	2	10	10	10	10	10	100	
	3	10	10	10	10	9	90	
12.5	1	10	10	10	10	10	100	96.7
	2	10	10	10	10	10	100	
	3	10	10	10	10	9	90	
25	1	10	5	1	0	—	0	6.7
	2	10	6	4	2	2	20	
	3	10	3	1	0	—	0	
50	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	

LC50 = 17.9 mg/L.

Laboratory mean =  $14.71 \pm 4.10$ .

Acceptable sensitivity.

**REFERENCES**

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U.S. EPA. 1993. Methods for measuring acute toxicity of effluents to freshwater and marine organisms, 4th ed. EPA 600/4-90/027F, August, 1993.